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Microvascular Regeneration in Meshed Skin Transplants after severe Burns

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Abstract

Function of the skin lymphatics as well as blood perfusion of a meshed transplant is crucial for the healing. The lymphatic regeneration and arterial perfusion of skin transplants after severe burns of the extremities had been studied in 8 patients by microlymphography, Laser Doppler Perfusion Imaging and transcutaneous oxygen pressure measurements 1, 6 and 18 months after transplantation.

One month after transplantation only fragmented as well as many giant lymphatic skin vessels were present in the transplant. After 6 months a normal lymphatic network had developed in all grafts. The extension of the dye in the lymphatics decreased from 4.5mm (0-16) at 1 month to 3.0mm (1-6) after 18 months indicating improved lymph drainage capacity. The permeability of the lymphatics in the graft was normal. After 1 month median laser flux in the transplant was 155.6% (105-246%) of the normal skin but normalized within 18 months. In contrast, TcPO₂ increased from 44mmHg (21-47) to 55mmHg (50-76).

In meshed transplants used to cover severely burned skin morphological and functional normal lymphatics develop within 6 months and the initially increased laser flux due to inflammatory reaction normalizes. Our results provide important insights in the healing process of skin transplants after burn.

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Deep burn injuries involving the entire dermis or extend beneath the dermis into fat, muscle and bone show a poor and prolonged wound healing taking weeks and even months if they are managed conservatively. This long time stretch involves a high risk of systemic disease with multiorgan failure and local infection, post-burn-oedema and cicatricial contracture. Therefore the preferred approach is a combination of excision and grafting[1]. Reduction of the size of skin-graft donor sites can be accomplished by meshed grafts, allowing the skin to expand by up to six times of the original area.

Different factors influence the normal wound healing. Multifunctional cytokines such as the vascular permeability factor / vascular endothelial growth factor (VPF / VEGF) induce an angiogenic as well as a strong lymphangiogenic response[2]. From day 5 of wound healing onward VEGF-receptor-3 (VEGFR-3) positive vessels can be observed by immunohistochemistry in the granulation tissue. These vessels appear to sprout from pre-existing VEGFR-3-positive lymphatic vessels at the wound edge[3]. This sprouting and the formation of anastomosis between the skin microlymphatic networks around the scar in replanted fingers had been convincingly demonstrated by microlymphography[4]. On the other hand in split-skin grafts of patients with venous ulcers there was almost no regeneration of lymphatics even years after the transplantation[5]. The wound-ground appears to play an important role in the regeneration of lymphatic vessels.

The flux was the subject of several studies whose results suggested that the LDPI is a suitable means of assessing tissue perfusion in healing burns. These studies demonstrated that deep dermal and full thickness burns have perfusion levels less than those of normal skin and that for this case surgical treatment is beneficial. In contrast, superficial burns have elevated perfusion levels as much as three to five

times the level of normal skin[6-9].

TcPO₂ measurement is used routinely for monitoring of “skin graft take”[10, 11]

Only little is known about the regeneration and function of lymphatic vessels and the early microvascular perfusion in meshed grafts on burn wounds. The aim of this study is to evaluate lymphatic regeneration and arterial perfusion in skin transplants in patients with severe burns.

Methods

Patients

In this prospective, non-randomized study patients with deep burn injuries grade II and grade III on the extremities treated with a meshed skin graft had been included from January 2006 to December 2008. The therapeutic management in all patients consisted of an excision of the burn eschar followed by a grafting with a meshed skin transplant. At the time of the study all skin grafts were taken.

In all patients the lymphatic regeneration in the skin graft was studied by fluorescence microlymphography; the blood perfusion of the grafted skin area was examined by laser doppler perfusion imaging and transcutaneous oxygen tension measurement.

The study had been approved by the local ethical committee (EK 1711) and all patients gave written informed consent.

Fluorescence Microlymphography

The technique of fluorescence microlymphography has been described in detail[12]. Using a steel microcannula with a tip diameter of 0.2 mm (Arnold Bott, Zurich, Switzerland) connected to a microsyringe (Hamilton, Bonaduz, Switzerland), the amount of 10µl of a sterilized 25% solution of FITC-(fluorescein-isothiocyanate) dextran with a molecular weight of 150000 (TdB Consultancy AB Viridings Allé 28, SE-754 50 Uppsala, Sweden) is injected into the subepidermal layer of the skin. Since large molecules are exclusively drained by the lymphatic system, the fluorescent dextran molecules move into the initial lymphatics and can be visualized by a fluorescence video microscopy system. Basically, it consists of an incident-light fluorescence microscope (Leica, Heerbrugg, Switzerland), a 3-CCD video camera (model DXC-930P, Sony, Tokyo, Japan) with a camera adapter and sensitivity on

automatic control (CMA-D2, Sony), a timer (VTG-22) and scale marker (IV-600; both from For-A-Company, Tokyo, Japan), a monitor (Picture Monitor model PM 171T, Ikegami Tsushinki, Tokyo, Japan) and a recorder. The microscope is equipped with 1.0/0.04, 2.5/0.08, 6.3/0.20 and 10/0.25 planar objectives (Leica), which allow a magnification of $\times 24$, $\times 62$, $\times 165$ and $\times 240$, respectively, on the monitor. The fluorescence excitation filter works at 450-490 nm and the barrier filter at 515 nm.

Morphology of lymphatic vessels in the skin transplant

The following parameters have been evaluated off-line from the videotape 10 minutes after dye injection: Presence of visualized lymphatic meshes; fragmentation of the superficial lymphatic network (defined as network with interruptions of the meshes); cutaneous backflow (defined as abnormal retrograde flow from deep to cutaneous lymphatics away from the main network), maximal extension of the fluorescent macromolecules into the lymphatic network in the lateral, proximal, medial, and distal direction as a measure for lymphatic drainage capacity into deeper channels and diameter of single lymphatic microvessels. Giant lymphatic skin vessels are defined as lymphatic capillaries with a diameter of more than $56.3\mu\text{m} \pm 9\mu\text{m}$ [12].

Lymphatic Permeability

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microlymphography 10 minutes after dye injection were digitalized and colours were transformed into black and white (synedra view and image view software, synedra information technologies GmbH, Innsbruck, Austria). Single images of well-delineated lymphatic capillaries as far away as possible from the dye depot were chosen. A 10x10 pixel region of interest was localized over the brightest point in the lymphatic capillary and 0.5mm away from the capillary wall in the interstitial tissue. The light intensity was measured three times by the image processing and analysis software ImageJ (Andreas Jahnen, Luxembourg). Light intensity was expressed in arbitrary density units (DU) and the light intensity in the vessel defined as 100%. The difference from intraluminal to interstitial given as percentage was compared to normal skin and changes over time were noted.

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The polarographic technique of transcutaneous oxygen tension measurement has been described in detail[14]. Transcutaneous oxygen values were obtained with the electrode (Radiometer, Copenhagen, DK) set at a temperature of 44°C to produce local hyperemia. The measurement result is indicated in mmHg and is a marker of the nutritive microcirculation of the skin.

Laser Doppler Perfusion Imaging (LDPI)

Laser doppler perfusion imaging (LDPI) is a newer method to determine the local distribution of flux over a skin area without direct contact with the tissue surface. The laser light is emitted from the source in a box placed approximately 20cm above the skin [15, 16]. Flux is a relative measure of the microvascular flow, represents the product of speed and number of cells and is expressed in arbitrary perfusion units

(PU). The LDPI has an average measurement depth in human skin of about 1-1.5mm and therefore the nutritive and thermoregulative microcirculation is examined[16, 17]. In this study a PIM 2.0 Laser Doppler Perfusion Imager (Periscan PIM II, Perimed AB Järfälla (Stockholm), Sweden) was used, with a low power Helium-Neon laser beam (1mW, 670nm). The LDPI provides colour coded two dimensional images of the blood flow through the investigated tissue. In the pictures the lowest perfusion is represented by a dark blue colour, when perfusion increases the colors change from light blue, green, yellow, orange to red. These images were analyzed with the LDPIwin System Software (Perimed AB Järfälla, Stockholm, Sweden). The same region of interest in the subsequent two LDPI images of a certain skin area was selected and the median flux was calculated. The flux of the normal skin was defined as 100%.

Investigational Protocol

All measurements were performed in the center of the meshed graft and in the normal skin about 10cm surrounding the graft three to six weeks, six months and 18 months after the skin transplantation. During the procedure patients with the burn injury on the leg were in supine position with the leg at heart level and patients with the injury on the upper extremity in sitting position with the arm at heart level. The temperature of the air conditioned examination-room was maintained between 22 and 24°C as it is recommended [7].

Statistical Analysis

Statistical analysis was performed using the statistical program (SPSS Inc., IBM Company, Chicago, IL). Results are presented as median and ranges (in brackets).

Results

Patients

Eight patients (5 male, median age 48.5, range 35-69 years) with deep burn injuries grade II and grade III had been included in the analysis. The burn injuries were located on the leg in two patients and on the upper extremity in six patients. The median age of the split-skin grafts at the time of inclusion was 29 days (22-35). The size of the grafts was between 20 to 60cm².

Fluorescence Microlymphography

One month after transplantation only fragments of lymphatic skin vessels had been present in the transplants. Already after one month and even more so after six months a certain number of lymphatic meshes have been detected, and after 18 months normal meshes were present in all patients.

In the first months after grafting many giant lymphatic skin vessels were found in all grafts, whereas after 18 months they had not been detected in any graft. Accordingly the median lymphatic capillary diameter in the split skin graft decreased from 101µm (71-126) one month after transplantation to 52µm (37-60) after 18 months. The median capillary diameter in the normal skin of the forearm, upper arm and thigh were at one month 60.0 µm (41-90), at six months 63.5 µm (63-63.5) and at 18 months 71 µm (63-119) respectively. Cutaneous backflow had not been present at any time. At one month median extension of the dye in the skin graft was 4.5mm (0-16.0) and decreased slightly after six months to 2.5mm (1.5-6.0). No further decrease was observed after 18 months. There was no difference to the normal skin area (3.5mm, 2.5-5.0), (figure 3).

The difference of the median light intensity between the lymphatic vessel and the interstitial space did not differ in skin grafts compared to normal skin at all three time

points. It was 44.5% in the grafts and in the normal skin at one month, 37.7% vs. 39.1% at six months, and at both sites 43.7% at 18 months indicating normal permeability of the developing microlymphatics already in the early phase of transplant healing.

Laser Doppler Perfusion Imaging (LDPI)

After one month median flux in the transplant was 155.6% (105-246) and decreased after six months to 123% (98-154) of the flux in normal skin. After 18 months a further decrease of the median flux to 90.5% (47.3-110.7) was measured (figure 4).

Transcutaneous Oxygen Tension Measurement (TcPO₂)

Median TcPO₂ in the meshed graft at one month was 44mmHg (21-70), it increased after six months to 50.5mmHg (50-70), which corresponds to the values in the normal skin (55mmHg, 41-60) and didn't change 18 months after transplantation.

Discussion:

This is the first study demonstrating the regeneration and function of skin lymphatics and blood microvessels of in-vivo meshed transplants after deep burns in humans. Microlymphography revealed a fast regeneration of sufficiently working lymphatic skin vessels in meshed skin transplants after deep burn injuries of the extremities. Normal microlymphatic vessels were found already after six months and even more so 18 months after transplantation. In the initial postoperative phase, giant lymphatic vessels had developed. After six months the diameter of the visualized lymphatic microvessels in the transplant was normal. The dye extension in the superficial lymphatic network decreased after six and 18 months and no cutaneous backflow was observed. These are indicators that the underlying lymphatic precollectors connected to the skin transplant and worked sufficiently. Furthermore, we were able to demonstrate that the permeability of the lymphatics in the skin grafts and in the normal skin was almost identical already one month after the transplantation. Despite the fact that in the first month giant lymphatics had developed, their permeability was not increased. This was also reflected by the absence of a clinically manifest edema of the graft. In summary, the lymphatic microvessels in meshed transplants covering severely burned skin show a very fast regeneration and their morphology as well as their function normalize in the first six months. This is not only of great importance for the healing of the transplant but also regarding infection and further function of the extremity. An earlier study from our lab has shown that this regeneration does not occur in meshed skin transplants covering venous ulcers [5]. This reflects the importance of the wound-bed in the regeneration of lymphatic vessels. In burn injuries, an excision of the eschar to create a viable wound bed is usually performed, whereas in venous ulcers, persistent chronic inflammation and microvascular dysregulation may be associated with an unfavourable wound bed.

In our study, the arterial perfusion in the transplant measured by laser flux was initially increased due to inflammatory reaction and increased vascularisation during wound healing. Perfusion was reduced after six and even more after 18 months, reflecting normalization of the transplant. Barachini et al. could demonstrate a similar blood flow pattern with higher flux in the grafts than in controls during the first three to ten weeks[18]. In contrast to the laser flux, TcPO₂ increased slightly over time due to regeneration of the normal capillary bed, reduction of interstitial edema and decreased inflammation leading to reduced metabolism. This corresponds with the third and final phase of the take of skin grafts. This phase of organization lasts over months and is characterized by TcPO₂ levels in the graft approaching the TcPO₂ levels of the skin. The graft matures and becomes less active metabolically than during the first weeks after grafting, where imbibition and revascularization takes place and TcPO₂ values are very low[11]. Our results indicate that the laser flux in skin grafts reflects the revascularisation and inflammation, whereas the TcPO₂ has the potential for indicating graft maturity.

Conclusion:

In meshed skin transplants used to cover severely burned skin, rapid regeneration of sufficiently working lymphatic skin vessels and normalization of arterial perfusion could be demonstrated. With several non-invasive or minimally invasive methods, microcirculation of the transplant can be monitored and our data allows better characterization of potential problems in the healing process of a skin graft.

Acknowledgment

We thank Mrs G. Gitzelmann for the technical support in microlymphography.

Conflict of interest statement

None to declare.

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Legends for illustrations:

Figure 1: Fluorescence microlymphography in a meshed skin graft (magnification 25x): Fragments of capillaries were present one month after graft transplantation, however after six months an almost normal lymphatic network has developed.

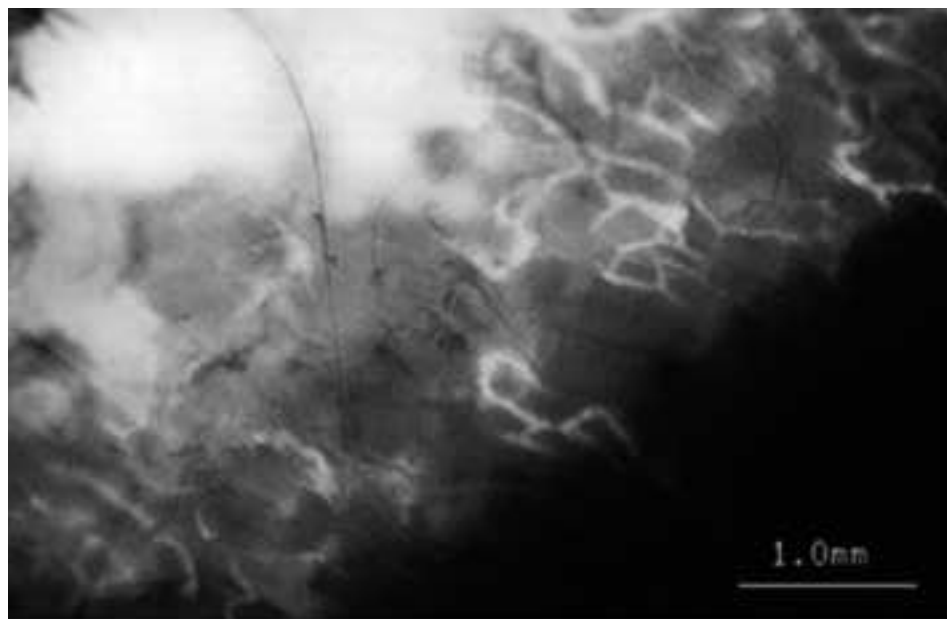
Figure 2: Median diameter of lymphatic capillaries in the skin grafts (shaded boxes) has normalized after six months (normal skin: white boxes).

Figure 3: Median extension of dye in lymphatic networks in the skin grafts (shaded boxes) and in normal skin (white boxes): after six months the extension of dye was more homogenous in all grafts.

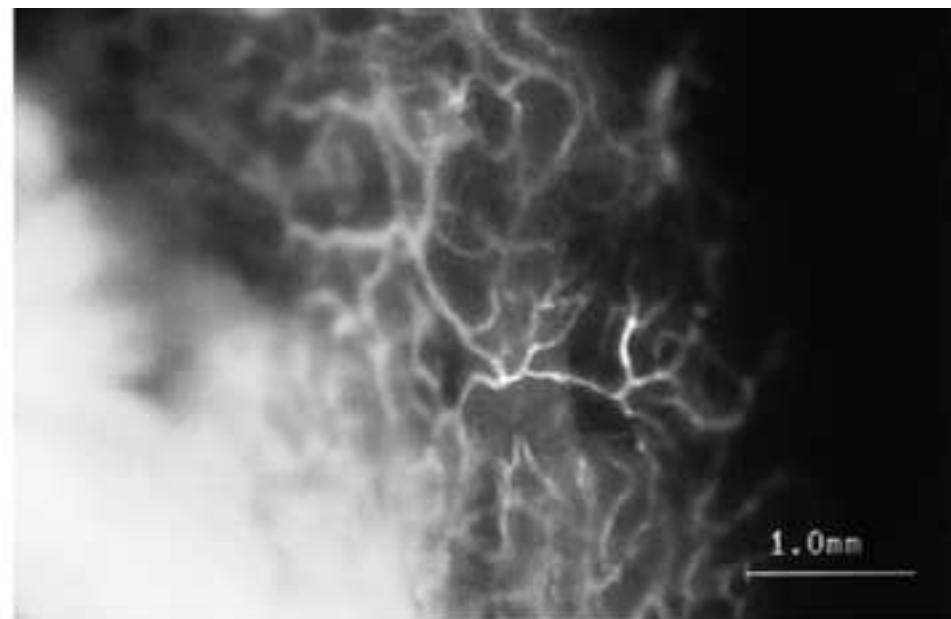
Figure 4: Difference of median flux between normal skin and meshed graft skin decreased during the follow up.

Figure

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1month



6 months

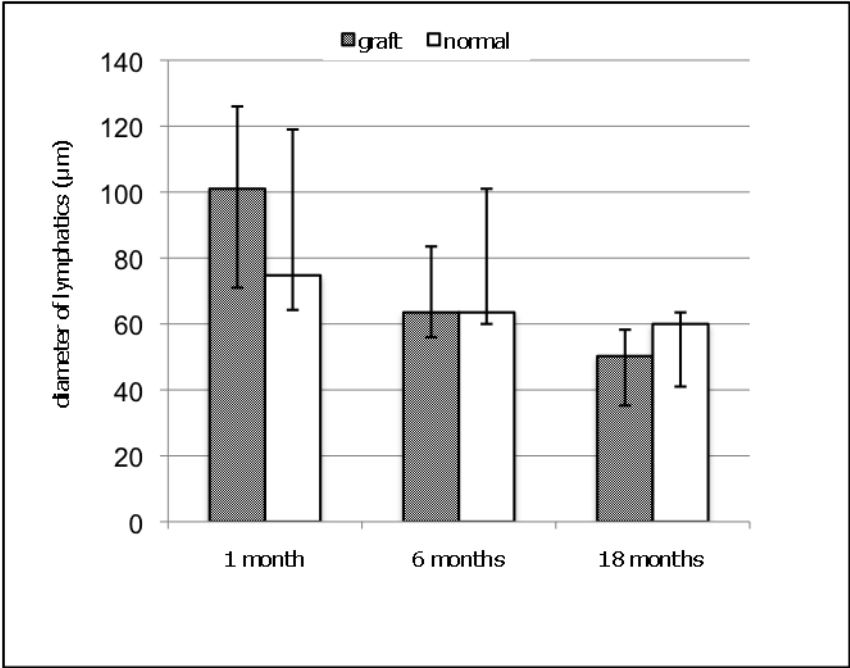


Fig. 2

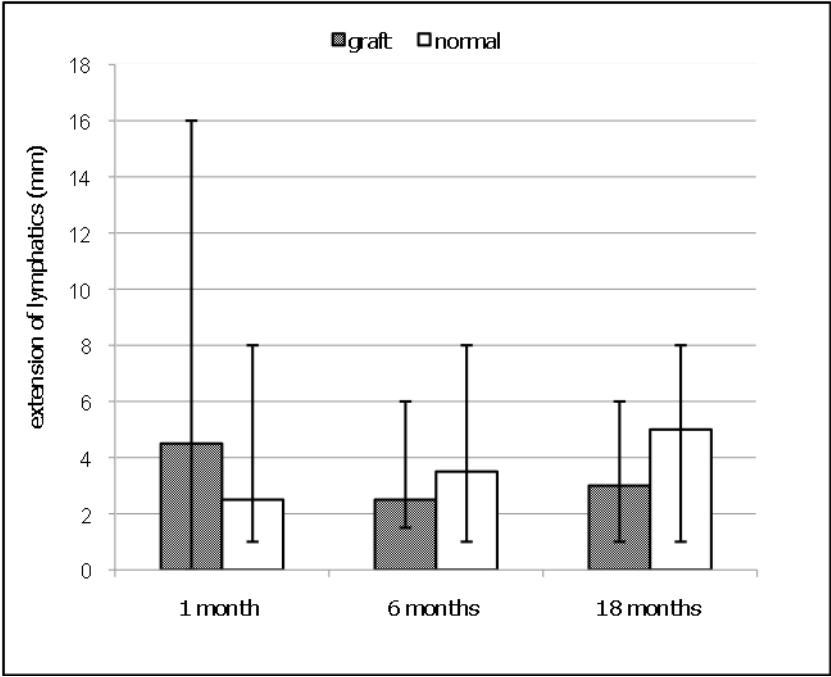


Fig. 3

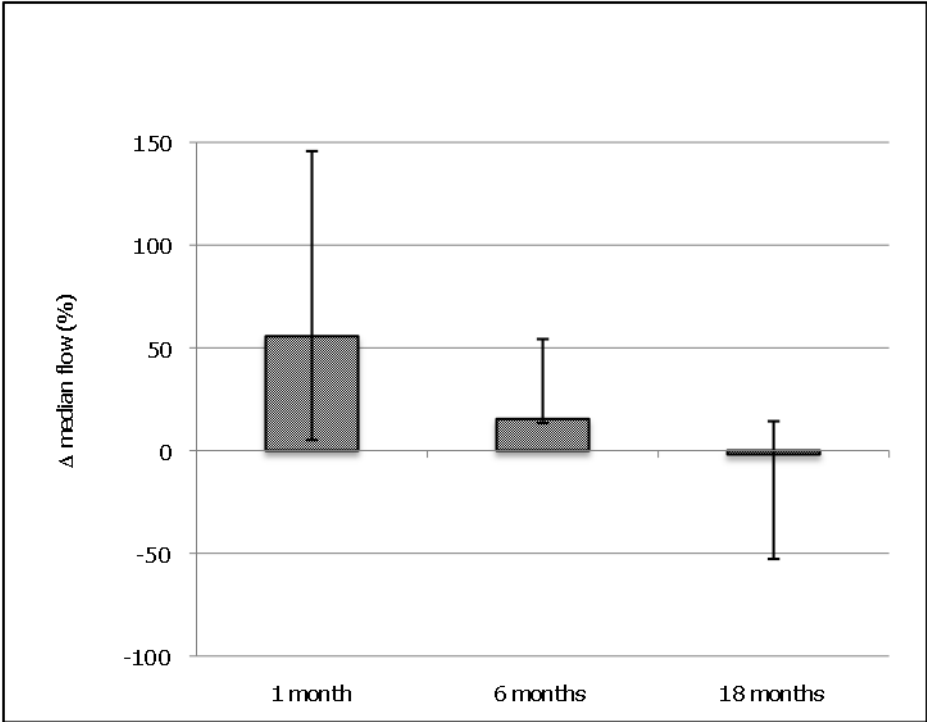


Fig. 4

Microlymphatic Regeneration in Meshed Skin Transplants after severe Burns

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Laser Doppler Perfusion Imaging (LDPI)

Laser doppler perfusion imaging (LDPI) is a newer method to determine the local distribution of flux over a skin area without direct contact with the tissue surface. The laser light is emitted from the source in a box placed approximately 20cm above the skin [15, 16]. Flux is a relative measure of the microvascular flow, represents the

product of speed and number of cells and is expressed in arbitrary perfusion units (PU). The LDPI has an average measurement depth in human skin of about 1-1.5mm and therefore the nutritive and thermoregulative microcirculation is examined[16, 17]. In this study a PIM 2.0 Laser Doppler Perfusion Imager (Periscan PIM II, Perimed AB Järfälla (Stockholm), Sweden) was used, with a low power Helium-Neon laser beam (1mW, 670nm). The LDPI provides colour coded two dimensional images of the blood flow through the investigated tissue. In the pictures the lowest perfusion is represented by a dark blue colour, when perfusion increases the colour changes from light blue, green, yellow, orange to red. These images were analyzed with the LDPIwin System Software (Perimed AB Järfälla, Stockholm, Sweden). The same region of interest in the subsequent two LDPI images of a certain skin area was selected and the median flux was calculated. The flux of the normal skin was defined as 100%.

Investigational Protocol

All measurements were performed in the center of the meshed graft and in the normal skin about 10cm surrounding the graft three to six weeks, six months and 18 months after the skin transplantation. During the procedure patients with the burn injury on the leg were in supine position with the leg at heart level and patients with the injury on the upper extremity in sitting position with the arm at heart level. The temperature of the air conditioned examination-room was maintained between 22 and 24°C as it is recommended [7].

Statistical Analysis

Due to the very small sample size no statistical testing was performed. Descriptive statistic results are given as median and ranges. Statistical analysis was performed

using the statistical program (SPSS Inc., IBM Company, Chicago, IL).

Results

Patients

Eight patients (5 male, median age 48.5, range 35-69 years) with deep dermal and full thickness burn injuries had been included in the analysis. The burn injuries were located on the leg in two patients and on the upper extremity in six patients.

The median age of the split-skin grafts at the time of inclusion was 29 days (22-35).

The size of the grafts was between 20 to 60cm². The expansion rate of the meshed grafts was not more than 1:1.5.

Fluorescence Microlymphography

One month after transplantation only fragments of lymphatic skin vessels had been present in the transplants. Already after one month and even more so after six months a certain number of lymphatic meshes have been detected, and after 18 months normal meshes were present in all patients.

In the first months after grafting many giant lymphatic skin vessels were found in all grafts, whereas after 18 months they had not been detected in any graft. Accordingly the median lymphatic capillary diameter in the split skin graft decreased from 101µm (71-126) one month after transplantation to 52µm (37-60) after 18 months. The median capillary diameter in the normal skin of the forearm, upper arm and thigh were at one month 60.0 µm (41-90), at six months 63.5 µm (63-63.5) and at 18 months 71 µm (63-119) respectively. Cutaneous backflow had not been present at any time. At one month median extension of the dye in the skin graft was 4.5mm (0-16.0) and decreased slightly after six months to 2.5mm (1.5-6.0). No further decrease was observed after 18 months. There was no difference to the normal skin area (3.5mm, 2.5-5.0), (figure 3).

The difference of the median light intensity between the lymphatic vessel and the

interstitial space did not differ in skin grafts compared to normal skin at all three time points. It was 44.5% (38.8-62.1) in the grafts and in the normal skin also 44.5% (21-72) at one month. At six months 37.7% (33-54) in the grafts and 39.1% (33-53.7) in normal skin, and at both sites 43.7% at 18 months indicating normal permeability of the developing microlymphatics already in the early phase of transplant healing.

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After one month median flux in the transplant was 155.6% (105-246) and decreased after six months to 123% (98-154) of the flux in normal skin. After 18 months a further decrease of the median flux to 90.5% (47.3-110.7) was measured (figure 4).

Transcutaneous Oxygen Tension Measurement (TcPO₂)

Median TcPO₂ in the meshed graft at one month was 44mmHg (21-70), it increased after six months to 50.5mmHg (50-70), which corresponds to the values in the normal skin (55mmHg, 41-60) and didn't change 18 months after transplantation.

Discussion:

This is the first study demonstrating the regeneration and function of skin lymphatics and blood microvessels of in-vivo meshed transplants after deep burns in humans. Microlymphography revealed a fast regeneration of sufficiently working lymphatic skin vessels in meshed skin transplants after deep burn injuries of the extremities. In the initial postoperative phase, giant lymphatic vessels with a median diameter of $101\mu\text{m}$ (71-126) had developed. However, after six months and even more so after 18 months, the diameter of the lymphatic microvessels in the transplant was already normal with a median diameter of $52\mu\text{m}$ (37-60). Similarly, the dye extension in the superficial lymphatic network of the grafts normalized within six months to 2.5mm (1.5-6.0) compared to 4.5mm (0-16.0) at 1 month. No cutaneous backflow was observed. These are indicators that the underlying lymphatic precollectors connected to the skin transplant and worked sufficiently. Furthermore, we were able to demonstrate that the permeability of the lymphatics in the skin grafts and in the normal skin was almost identical already one month after the transplantation. Despite the fact that in the first month giant lymphatics had developed, their permeability was not increased. This was also reflected by the absence of a clinically manifest edema of the graft.

In summary, the lymphatic microvessels in meshed transplants covering severely burned skin show a very fast regeneration. Their morphology as well as their function normalize in the first six months. This might explain the high rate of meshed skin graft take in burn injuries, since the normal function of lymphatic circulation is crucial for wound healing, prevention of oedema and infection. In contrast lymphatic regeneration does not occur in meshed skin transplants covering venous ulcers as an earlier study from our lab has shown [5]. The lack of lymphatic regeneration in

addition to a skin hypoxia in chronic venous insufficiency might explain the rather high recurrence rate of venous ulcers despite skin grafting and the increased risk of skin graft failure in venous ulcer [18] [19].

Furthermore our findings might reflect the importance of the wound bed in the regeneration of lymphatic vessels. In burn injuries, an excision of the eschar is usually performed to create a viable wound bed inducing a fast [microlymphatic vascular regeneration](#), whereas in venous ulcers, persistent chronic inflammation and microvascular dysregulation may be associated with an unfavourable wound bed and therefore with a lack of [microlymphatic vascular regeneration](#).

[To allow some degree of standardisation and for cosmetic reasons, we never grafted the burn wounds of our patients with an expansion rate of more than 1:1.5. It would, however, be interesting to compare microlymphatic vascular regeneration for different expansion rates in subsequent studies.](#)

In our study, the arterial perfusion in the transplant measured by laser flux was initially increased due to inflammatory reaction and increased vascularisation during wound healing. Perfusion was reduced after six and even more after 18 months, reflecting normalization of the transplant. Barachini et al. could demonstrate a similar blood flow pattern with higher flux in the grafts than in controls during the first three to ten weeks[20]. In contrast to the laser flux, TcPO₂ increased slightly over time due to regeneration of the normal capillary bed, reduction of interstitial edema and decreased inflammation leading to reduced metabolism. This corresponds with the third and final phase of the take of skin grafts. This phase of organization lasts over months and is characterized by TcPO₂ levels in the graft approaching the TcPO₂ levels of the skin. The graft matures and becomes less active metabolically than during the first weeks after grafting, where imbibition and revascularization takes

place and TcPO₂ values are very low[11]. Our results indicate that the laser flux in skin grafts reflects the revascularisation and inflammation, whereas the TcPO₂ has the potential for indicating graft maturity.

Conclusion:

In meshed skin transplants used to cover severely burned skin following its excision, rapid regeneration of sufficiently working lymphatic skin vessels and normalization of arterial perfusion could be demonstrated. With several non-invasive or minimally invasive methods, microcirculation of the transplant can be monitored and our data allows better characterization of potential problems in the healing process of a skin graft.

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